Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Genetic confirmation for morphological identification of *Stilesia globipunctata* in camel in Iraq

H.H. Abed, A.I. Fadhil, A.R. Alhaboubi and A.A. Farj

Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Article information

Article history:
Received December 14, 2022
Accept March 29, 2023
Available online June 18, 2023

Keywords:
Arabian
Camel
Stilesia spp
Cestode

Correspondence:
A.R. Alhaboubi
arussul@covm.uobaghdad.edu.iq

Abstract

Camels (*Camelus dromedaries*), similar to other ruminants, are infected by the Anoplocephalidae family belonging to the class cestode causing mild to severe illness. This study utilized a conventional PCR assay to confirm *that Stilesia spp*. is morphologically identified. Slaughtered camel intestines from the Al-Najif abattoir in the central part of Iraq were morphologically examined for *Stilesia* spp. Applied a PCR and genetic analysis for twenty adult worms. The presence of *Stilesia* spp. adult worms were initially identified through morphological characterization. The scolex and strobili were found in the intestine lumen and its nodules. All the worms' specimens were identified as a *Stilesia* genus, and PCR amplified their partial DNA fragment on the location of the ITS2, 5.8S rDNA gene. Camels infected with *Stilesia* spp. found in eviscerated camel carcasses in south Iraq, and twenty isolates of *Stilesia globipunctata* molecular data have been recorded an accession numbers OM221663- OM221682 in the NCBI.

DOI: 10.33899/ijvs.2023.137262.2664,

Introduction

Arabian camel (*Camelus dromedaries*) is well-adapted to extreme heat and dry conditions (1). In addition to the use for transportation recently, it has emerged for festivals and events, tourism, and racing in Arab gulf countries (2). Parasites, including Cestoda, affected camels as other ruminants causing mild to severe illnesses and significant economic loss (3-7) *Stilesia* spp. (Rivolta, 1874) is a neglected ruminant parasitic (cestode) infection that invades the small intestine of camels (definitive host) in tropical and subtropical areas (8,9). *Stilesia globipunctata* belong to the Anoplocephalidae family of Cestoda. The scolex and proglottids of the adult parasite can grow up to 60 cm in length, and because of the prolonged immune reaction, they penetrate the mucosal membrane and develops many nodules in the duodenum (10). *Stilesia globipunctata* has been recorded in sheep and goats, and the heavy parasitic load with *S.globipunctata* can result in death (11). Morbidity in ruminants ranged between 30 to 65 percent (12). Oribatid mites act as an intermediate host for the larval stage (cysticercoid), which develops inside the mite, completing the life cycle (13-15). In general, the intermediate hosts for *S.globipunctata* are *Scheloribates undica* and *Erythraeus* spp. *Stilesia hepatica* is a species affecting sheep and goats and is more prevalent than S. *globipunctata* (16-18). Morphological traits can be used for parasite diagnosis and quantification (19).

In this study, the investigation includes camels slaughtered in the center of Iraq, and due to the limited morphological information and lack of molecular data, molecular techniques were used to identify this parasite by targeting the ITS gene. The study utilizes conventional PCR assays to identify worm samples of the genus at the species level.

Materials and methods

Ethical approve

The project approved and granted through the local committee of animal care at college of veterinary medicine, University of Baghdad No, 706 dated at 23/3/2022.

Samples collection

The study applied fifty of the camels slaughtered for human consumption in Al Najif province abattoir, central Iraq, from early April 2022 to September 2022. The abattoir facility served the city and the surrounding arias 31°59'46" North, 44°18'53" East which has tropical dry, hot weather with 25-49°C. Adult animals' small intestine samples were carefully dissected and cut longitudinally to examine the mucosal surface for any intact tapeworms. The identified adult worms were collected carefully to prevent damage and washed with a physiological saline solution in 70% ethanol. The collected worms were transferred for further processing at the parasitology department laboratories in the college of veterinary medicine at the University of Baghdad. Camel's intestine, the duodenum, and the jejunum were microscopically examined for the presence of *Stilesia* species.

Morphological examination

The collected worms were carefully washed, pressed between two slides, fixed, and then identified (19). Light microscope examination was maintained under its integrity image and scanning and morphometric data were recorded using the Olympus microscope (Olympus America, Inc., Center Vally, PA). Species identification was made according to the principles described (10,17).

DNA extraction and amplification

Collections of twenty adult worms of *Stilesia* spp. from camels, primarily identified by morphology, were applied for DNA extraction according to the kit manufacturing interaction of (EasyPure® Genomic DNA Kit.) Electrophoresis on 2% agarose was done to confirm the integrity and purity of the extracted DNA. The DNA concentration was determined by spectrophotometers (NanoDropND-100 Spectrophotometer, wellington, DE, USA). The DNA was stored at -20℃ for future application.

Primers and polymerase chain reaction

The common DNA fragment targeted to be a molecular marker for Cestoda in identification, and classification is the 18S RNA gene. For *Stelisia* spp. The ITS1-5.8S- ITS2 region sequence is based on data sequences available on the NCBI. A Forward primer 5′- GTTTACAAACTAC CACCACGGATCG -3′ Reverse primer 5′- CTGATTACGT CCCTGCCC TTTG -3. The primers were used according to the reference sequence data with the (accession no. MW601833 (20) and OM296990. The extracted DNA subjected to conventional amplification reaction (PCR mix and amplification profile) was performed according to manufacturer's instructions (EasyTag® PCR super Mix, as111).

Polymerase chain reaction master mix component consists, of 2x Easy TagPCR SuperMix by volume 12.5 µL to reach 1X final concentration with 10 µM for both Forward and Reverse Primers (1.0 µM). The templet DNA ~100 ng was added by 2 µL to mixture. Thermocycler conditions are set for the 35 amplification cycles start with 94°C (3min.) for both Initial denaturation and denaturation. The and primers annealing at 62°C in 15 s and the final extension for 5min. at the 72°C.

Sequences analysis

The obtained amplicons sequences for 20 worm samples represent a partial DNA fragment of approximately 980 bp flanking by the selected PCR primers, previously submitted for Sanger sequences. The PCR products were purified using an INTERON kit and analyzer (Macrogen) using terminator cycle sequencing and compared by Base Local Alignment Search Tool (nucleotide-nucleotide) with those available in GenBank™ (21). The row data was edited with MEGA 7 (22). Comparative molecular analysis for the identity of a Neighbor-Joining method was contacted using the default setting.

Agarose gel electrophoresis

Amplicons generated from the PCR reactions were visualized through 1% agarose gel stained by ethidium bromide (concentration, 0.5 µg/ml) of gel sol. All the bands were produced from DNA products amplified proximately 980bp.

Results

Morphological finding and description

This study revealed the presence of *Stilesia* species adult worms in the small intestine of the camel host. The scolex and its close strobili were embedded in the intestine throughout nodules, leaving worm-tailed segments threadlike and free in the lumen. Tapeworm's scolex shape is quadrangular wider anteriorly and narrow posteriorly, and measures 0.7-1mm, in length and 0.6-1.2mm in breadth. The four suckers are large ovals arranged in two pairs, attached. The mature segment is broader than long, having only one set of reproductive organs in each segment, tests in two groups, 4-7 on each side, laterally margins are convex and measure ~0.4 mm and ~1.5 mm in length and breadth, respectively (Figure 1).

Molecular finding

Molecular characterization of the *Stilesia globipunctata* in the current study was based on a small subunit RNA gene of ribosomal DNA of 20 isolate sequencing of the *Stilesia* species among the total of 25 worms, examined microscopically, which identified as a *Stilesia* genus. Worms' genomic DNA of the selected isolates was successfully amplified by PCR on the ITS2, 5.8S rDNA gene location with approximately 980bp fragment size (Figure 2).

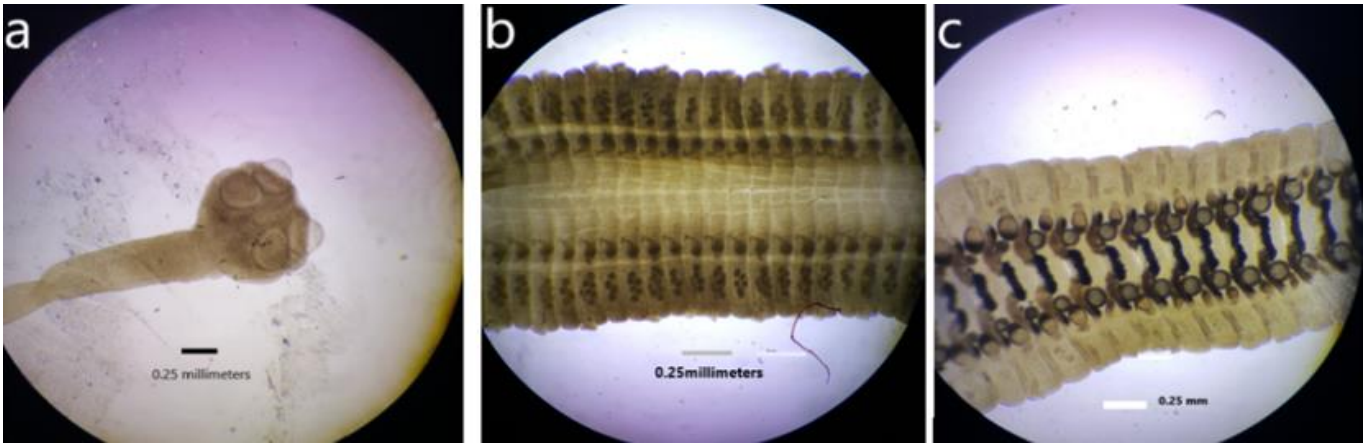

Figure 1: *Stilesia* spp. (a) Scolex large, oval with four suckers, (b) mature segment with 4 to 7 testes on each side, (c) graved segment. 40x.

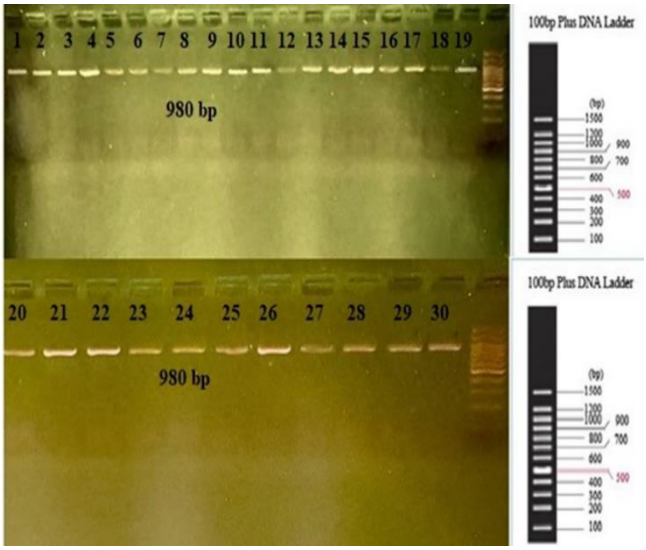

Figure 2: Gel electrophoresis for *S. globipunctata*, PCR product of 18s RNA, 980bp. Agarose 1%, at 70 volts.

Sequencing and phylogenetic analyses

The sequence data obtained in this study were compared with those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) analyses. The phylogenetic tree is constructed based on the analysis of partial SSU rRNA. The result revealed 99 similarities with *S. globipunctata* isolated EU051353.1 (Table 1).

The constricted phylogenetic tree was derived from ~770bp of the small subunit ribosomal RNA and ITS1gene of *Stilesia globijkpunctata* region sequences. The tree shows three major clades. The largest clade includes a cluster of the twenty identical isolates from our study, interrupting with sequence from GenBank® ID: EU051353.1, India. The second smaller clade, strongly supporting, enclosed a couple of sequences from India ID: MZ375460 & MZ37546. Finally, one strongly supported, out-grouped branch holding *S. globipunctata* with sequences ID: KC529650.1 from India (Figure 3).

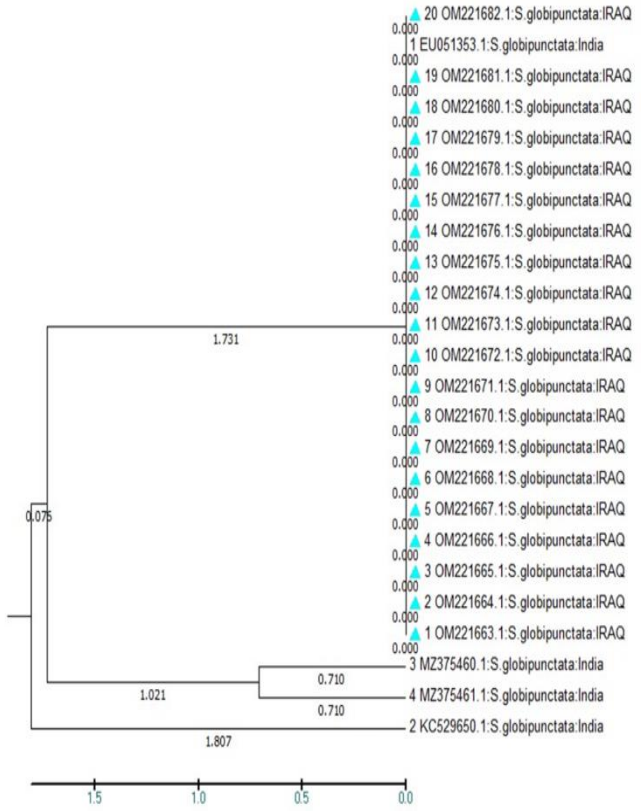

Figure 3: The phylogenetic tree was conducted using maximum likelihood capacity and branch lengths calculated pairwise (distance according to the UPGMA function) with boot strap1000 replicates. The light blue triangle indicates sequences found in the camels in this study.

Table 1: Single nucleotide polymorphisms for *Stilesia globipunctata* 18S ribosomal RNA gene

No.	Type	Location	Nucleotide	Seq. ID with compare	Seq. ID with submission	Identities
1	Transition Transition	383 414	G\A C\T	ID: EU051353.1	OM221663.1	99%
2	Transition	383	G\A	ID: EU051353.1	OM221664.1	99%
3	Transition Transition	383 414	G\A C\T	ID: EU051353.1	OM221665.1	99%
4	Transition	485	T\C	ID: EU051353.1	OM221666.1	99%
5	Transition Transition	383 414	G\A C\T	ID: EU051353.1	OM221667.1	99%
6	Transition	485	T\C	ID: EU051353.1	OM221668.1	99%
7	Transition	648	A\G	ID: EU051353.1	OM221669.1	99%
8	Transversion	699	C\A	ID: EU051353.1	OM221670.1	99%
9	Transversion	699	C\A	ID: EU051353.1	OM221671.1	99%
10	Transition Transversion	330 699	A\G C\A	ID: EU051353.1	OM221672.1	99%
11	Transition Transversion	330 699	A\G C\A	ID: EU051353.1	OM221673.1	99%
12	Transition Transversion	330 699	A\G C\A	ID: EU051353.1	OM221674.1	99%
13	Transition Transition	343 648	T\C A\G	ID: EU051353.1	OM221675.1	99%
14	Transition	600	C\T	ID: EU051353.1	OM221676.1	99%
15	Transition Transversion	600 754	C\T G\C	ID: EU051353.1	OM221677.1	99%
16	Transition	600	C\T	ID: EU051353.1	OM221678.1	99%
17	Transition	600	C\T	ID: EU051353.1	OM221679.1	99%
18	Transversion	699	C\A	ID: EU051353.1	OM221680.1	99%
19	Transition Transition	383 414	A\G C\T	ID: EU051353.1	OM221681.1	99%
20	Transition	485	T\C	ID: EU051353.1	OM221682.1	99%

Discussion

Generally, *Stilesia globipunctata* worm, listed as cattle Cestoda, although it has been found in other domestic and wild ruminants, including sheep and camels (23). Recently, and despite the limited studies on *Stilesia spp*., a new species has been described in small ruminants, mainly focusing on the characterization of adult worms' morphological features (24-26). In an early study in Iraq, *Stilesia vittata* was the first Cestoda tapeworm found in the small intestine of seven camels out of twenty-five examined slaughtered animals (18). Among the twenty-two *Stilesia* species reported in ruminants, *Stilesia vittata* previously registered in *Camelus dromedaries* which differs in having 5-9 testes each in two lateral groups, mature segments broader than long with unclear segmentation (25,26).

However, new molecular tool starts to be available veterinary parasitology practice in Iraq (27-29), PCR was still approved to be the more sensitive diagnostic method, valuable for speciation and identity between genetically similar parasitic isolates. The internal transcribed spacer ITS1/ITS2 genes previously used are reliable molecular markers for different nematode parasites in Iraqi camels (30-32). As previously reported in 18srRNA *genes* in India MZ375460.1, MZ375461.1 and KC529650.1 sequence version was not found among the twenty *S. globipuncta* isolates and was identical to each other. Nonetheless, the rDNA gene for *S. globipunctata* interestingly, the highest sequence 94-99% to 852 bp of *S. globipunctata* 18S rRNA gene, partial sequence, ITS1, complete sequence; and 5.8S rRNA gene, partial sequence), unpublished data in 2007 submitted from Andhra Pradesh, India. The diversity and taxonomy of the Iraqi Anoplocephalidae family of Cestoda class, infecting camel, are poorly known compared to the nematode family (33-36). Although *Stilesia vittata* rationally is the camel's intestine Cestoda found by Altaif (18), the molecular daintily results in clustering the obtaining DNA sequences of our study with *Stilesia globipunctata* from India. Unluckily, no sequencing DNA for the *S. vittata* species was submitted to the NCBI GenBank before, which

limits identity contrast. According to our knowledge, this is the first study to confirm the morphological finding of the species with the DNA sequences of *Stilesia* spp. from Iraq.

Conclusions

Twenty isolates of *Stilesia globipunctata* molecular data have been found and morphologically identified. The molecular characterization stated identity with previously recorded species, the advent of more molecular studies as the new pattern of the next-generation sequencing platform. The recognition receptors genes and their expression are very promising in ravel out. Since discovering and recording new *Stilesia* sp. nov., these genes' classification continues and is limited only to the morphological description.

Acknowledgment

Authors appreciate all kinds of assistance provided by slaughterhouse directors and workers.

Conflict of Interest

All authors declared no conflict of interest.

Reference

1. Hoter A, Rizk S, Naim HY. Cellular and molecular adaptation of Arabian camel to heat stress. Front Genet. 2019;10:588. DOI: 10.3389/fgene.2019.00588
2. Faye B. Special issue on "Some preliminary results of the PROCAMED project (Promotion of innovations in the camel sector for a sustainable developmentin The Mediterranean basin). Emir J Food Agric. 2015;27(4):318-327. DOI: 10.9755/ejfa.v27i4.19906
3. District MS, Ambedkar B. Morphological and molecular studies of *Moniezia sp.* (*Cestoda anaplocephalidea*) a parasite of the domestic goat *Capra hircus* (L.) in Aurangabad district. Zool. 2015;5(8):10-14. DOI: 10.36106/ijar
4. Abdulla RG, Mageed SN, Obed CE, Jumaa JA. Molecular characterization of fertile hydatid cysts from the liver of the sheep and cows and associated environmental influence factors. Iraqi J Vet Sci. 2020;34(2):321-327. DOI: 10.33899/ijvs.2019.126036.1213
5. Ali MJ, Atiyah WR, Al-Fatlawi MA, Khlaif SF. Genotypic analysis of tick's species infesting cattle in Al-Diwaniyah abattoir. Iraqi J Vet Sci. 2021;35(4):673-677. DOI: 10.33899/ijvs.2020.127772.1525
6. Jasim GA, Al-Fatlawi MA, Chaid ZH. Microscopic and molecular detection of *Babesia bovis* and *Babesia bigemina* in female camel from Al-Diwaniyah province, Iraq. Iraq J Vet Sci. 2022:37(1):61-64. DOI: 10.33899/ijvs.2022.133428.2226
7. Mohammed AA, Kadir MA. Estimation of some biochemical parameters and trace elements in sheep infested with *Taenia hydatigena* cysts in Sulaymaniyah province, Iraq. Iraqi J Vet Sci. 2020;34(1):39-44. DOI: 10.33899/ijvs.2022.131232.1932
8. Ndom M, Diop G, Quilichini Y, Yanagida T, Ba CT, Marchand B. Prevalence and scanning electron microscopic identification of anoplocephalid cestodes among small ruminants in Senegal. J Parasitol Res. 2016;1-9. DOI: 10.1155/2016/3937292
9. Sissay MM, Uggla A, Waller PJ. Prevalence and seasonal incidence of larval and adult cestode infections of sheep and goats in eastern Ethiopia. Trop Anim Health Prod. 2008;40:387-394. DOI: DOI:10.1007/s11250-007-9096-z
10. Soulsby EL. Helminths, arthropods, and protozoa of domesticated animals. 7th ed. London: Bailliere; 1982. 710 p. DOI: 10.1016/0035-9203(84)90110-X
11. Graber M, Gruvel J. The vectors of *Stilesia globipunctata* (Rivolta, 1874) of sheep. Rev Elev Med Vet Pay. 1967;20:261-271. DOI: 10.19182/remvt.7502
12. Louw JP. Diagnosis, distribution, and prevalence of *Stilesia globipunctata* (Rivolta 1874) in sheep in the Overberg region of the southern western Cape province. J S Afr Vet Assoc. 1995;66(4):244-266. [available at]
13. Asif Raza M, Iqbal Z, Jabbar A, Yaseen M. Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. J Helminthol. 2007;81(3):323-328. DOI: 10.1017/s0022149x07818554
14. Mohammed NH. Prevalence, morphological and biochemical study of larval stage *Coenurus cerebralis* of *Taenia multiceps* in sheep. Iraqi J Vet Sci. 2020;34(1):159-163. DOI: 10.33899/ijvs.2019.125660.1124
15. Sankar M, Bhatt M, Gowane GR, Narayanan K, Kumar M, Zahid AK, Saravanan BC, Allie I, Subramani V, Sharma AK, Prasad A. Effect of non-genetic factors on the prevalence of *Stilesia globipunctata* in the goat. Helminthol. 2016;53(2):186-190. DOI: 10.1515/helmin-2016-0011
16. Sazmand A, Joachim A. Parasitic diseases of camels in Iran (1931-2017): A literature review. Parasite. 2017;24(21):1-15. DOI: 10.1051/parasite/2017024
17. Tandon RS. Observations on the life history of the anoplocephaline cestode, *Stilesia globipunctata* Rivolta, 1874 (sub-family Thysanosominae): A common parasite of ruminants in India. Parassitol. 1963;5(3):183-188. [available at]
18. Altaif KI. Helminthes in camels in Iraq. Trop Anim Health Prod. 1974;6(1):55-57. DOI: 10.1007/BF02380750
19. Kaufmann J. Parasitic infections of domestic animals: A diagnostic manual. Basel: Springer; 1996. 24-35 pp. [available at]
20. Fraija-Fernández N, Waeschenbach A, Briscoe AG, Hocking S, Kuchta R, Nyman T, Littlewood DT. Evolutionary transitions in broad tapeworms (Cestoda: Diphyllobothriidea) revealed by mitogenome and nuclear ribosomal operon phylogenetics. Mol Phylogenet Evol. 2021;163(1):107262. DOI: 10.1016/j.ympev.2021.107262
21. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 1997;25(17):3389-3402. DOI: 10.1093/nar/25.17.3389
22. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Boil Evol. 2016;33(7):1870-1874. DOI: 10.1093/molbev/msw054
23. Borji H, Razmi Gh, Movassaghi AR, Naghibi A, Maleki M. A study on gastrointestinal helminths of camels in Mashhad abattoir, Iran. Iran J Vet Res. 2010;11(2):174-179. DOI: 10.22099/ijvr.2010.162
24. Rad MM, Hosseini SH, Rajabloo M, Nabian S, Sadeghian AG. Parasites of one-humped camel (*Camelus dromedarius*) in Iran: An abattoir study. J Camel Pract Res. 2015;22(2):261-264. DOI: 10.5958/2277-8934.2015.00043.0
25. Sampatrao JS, Devidasrao LA. A new tapeworm *Stilesia jadhavae* sp. nov. from the intestine of goat *Capra hircus* at Patoda tehsil of Beed district, India. World J Adv Res. 2020;7(1):222-226. DOI: 10.30574/wjarr.2020.7.1.026
26. Lakhe AD. A new species of cestode parasite, *Stilesia shindei*, from *Ovis bharal* at Parli. Life Sci Bull. 2018;15(1):59-61. DOI: 10.1007/s11250-007-9096-z
27. Abdullah WK, Maha FA. Caspase 9 gene expression and caspase 9 protein in chronic myeloid leukemia in Iraq. Iraqi J Agri Sci. 2022;53(5):994-1002. DOI: 10.36103/ijas.v53i5.1613
28. Elsahookie MM, Cheyed SH, Dawood AA. An overview on mechanism of gene expression regulation. Iraqi J Agri Sci. 2021;52(2):454-460. DOI: 10.36103/ijas.v52i2.1307
29. Salih DM, Abbas AK. The prevalence of canine dipylidiasis in Baghdad city, Iraq. Iraqi J Vet Med. 2022:46(1):24–29. DOI: 10.30539/ijvm.v46i1.1314

التأكيد الجيني للصفات الشكلية التشخيصية لديدان الستليزيا في الجمال في العراق

هويدة هامل عبد، علي عيسى فاضل، عامر رسول فضل و أزهار علي فرج

فرع الطفيليات، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

يشابه الجمل العربي المجترات الأخرى، بإصابته بديدان الشريطية التي تنتمي إلى الديدان الشريطية مسببتا إصابة خفيفة إلى شديدة. هدفت الدراسة إلى استخدام فحص تفاعل البلمرة المتسلسل التقليدي لتأكيد الاصابة بالستليزيا والتي تم التعرف عليها شكليا. فحصت أمعاء جمال مذبوحة في محافظة النجف وسط العراق عيانيا لوجود أجناس الستليزيا. تم تطبيق فحص البلمرة المتسلسل وتحليل النتائج الجزيئية لعشرين من الديدان من جنس الستليزيا المتعرف عليها خلال التوصيف الشكلي بعد العثور على قطع الرأس والجسم في تجويف الأمعاء وعقيداتها. تم تحديد جميع العينات ابتداء على أنها جنس الستليزيا. تم تضخيم جزء الحمض النووي بها بواسطة تفاعل البلمرة المتسلسل في موقع ITS2، وشجرة التطور الوراثي للجين ٥,٨ الدنا الرايبوسومي إذ تم تأكيد الاصابة في ذبائح الإبل في العراق وتم تسجيل عشرين عزلة من البيانات الجزيئية في البنك الجيني تحت الأرقام التعريفية OM221682 -OM221663.

30. Fadhil AI, Abed HH, Fadel SR, Al-Zubaidi MS. Molecular diagnosis of nematode worms *Parabronema Skrjabini* in camels (*Camelus dromedaries*) In Iraq. Iraqi J Agric Sci. 2022;53(3):584-588. DOI: 10.36103/ijas.v53i3.1567
31. Faraj AA, Al-Amery AM. Microscopic and molecular diagnosis of *Ascaridia spp*. in domestic pigeons (*Columba livia domestica*) in Baghdad city, Iraq. Iraqi J Agric Sci. 2020;51(4):1220-1225. DOI: 10.36103/ijas.v51i4.1101
32. Al-Fatlawi MA. COX1 gene and ITS-2 region: A comparative study of molecular diagnosis of *Parabronema skrjabini* in camels (*Camelus dromedaries*), Al-Najaf province, Iraq. Iraqi J Vet Sci. 2022;36(1):77-84. DOI: 10.33899/ijvs.2021.129228.1634
33. Alhaboubi AR, Fadhil AI, Feidhel SR. Prevalence and molecular identification of *Nematodirus helvetianus* in camels in Iraq. Vet World. 2021;14(5):1299-1302. DOI: 10.14202/vetworld.2021.1299-1302
34. Al-Dahar AH, Al-Amery A. A prevalence of abomasal worms in sheep in Al-Kut province abattoir. Iraqi J Vet Med. 2015;39(2):38-41. DOI: 10.30539/iraqijvm.v39i2.175
35. Fadhil AI, Mohammed TH, Al-Zubaidi S. Identification of *Haemonchus longistipes* from camels (*Camelus dromedaries*) by PCR. J Vet Res. 2018;22(10):914-918. [available at]
36. Al-Fatlawi MA, Ismail YK, Ali MJ, Karawan AC, Ibadi IN. Molecular differentiation of Thysaniezia (Helictometra) Giardi and Moniezia species based on 18s rRNA gene in small ruminants. Iraqi J Vet Sci. 2021;35(1):105-108. DOI: 10.33899/ijvs.2020.126407.1313